Scientific paper

Lipase-Catalyzed Transesterification of (*R,S*)-1-Phenylethanol in SC CO_2 and in SC CO_2/Ionic Liquid Systems

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Abstract

Commercial immobilized lipase B from *Candida antarctica* (CALB) was successfully applied to catalyzing the transesterification of (*R,S*)-1-phenylethanol in supercritical carbon dioxide and in supercritical carbon dioxide/ionic liquid biphasic system. Firstly, the variables affecting the performance of CALB in transesterification reactions in supercritical carbon dioxide, such as CALB concentration, temperature and pressure, were studied. An increase in the conversion and in the reaction rate was observed as the CALB/substrate ratio, temperature and pressure, were increased from 4.3 to 19.9, from 40 °C to 80 °C and from 8 MPa to 10 MPa, respectively. Further increase in temperature from 80 °C to 120 °C and pressure from 10 MPa to 30 MPa resulted in lower conversion and lower initial reaction rate. Furthermore, different vinyl esters were used as acyl donors for CALB-catalyzed transesterification of (*R,S*)-1-phenylethanol in supercritical carbon dioxide. The highest initial reaction rate was attained with vinyl butyrate, although 50% conversion was attained faster when vinyl acetate was used as acyl donor.

Secondly, in transesterification of (*R,S*)-1-phenylethanol, performed in supercritical carbon dioxide/ionic liquid biphasic system, influence of concentration of 1-butyl-3-methylimidazolium tetrafluoroborate [bmim][BF_4] was studied. The addition of 50 mmol (70% w/w reaction mixture) of [bmim][BF_4] to the reaction system gave the best result in terms of transesterification rate.

Keywords: *Candida antarctica* lipase B, transesterification, (*R,S*)-1-phenylethanol, vinyl acetate, supercritical carbon dioxide, ionic liquids

1. Introduction

Up to now enzyme-catalyzed reactions have been performed in non-solvent systems and in different solvent systems, such as micro-emulsions[1], aqueous systems[2], organic solvents[3], and also in unconventional solvents, such as supercritical fluids (SCFs)[4] and ionic liquids (ILs).[5]

The use of SCFs as non-aqueous media for enzyme-catalyzed reactions has been widely studied during the last decade, due to their excellent recognized properties.[6–8] Among all SCFs, supercritical carbon dioxide (SC CO_2) is by far the most studied SCF. Its properties, such as density, dielectric constant, diffusivity, viscosity and solubility can be tuned by adjusting the pressure and temperature[9], which clearly distinguishes SC CO_2 from conventional solvents and which enables solvent effects to be examined without changing the kind of solvent. Furthermore, SC CO_2 is non-cancerogenic, non-toxic, non-mutagenic, non-flammable and thermodynamically stable, it has near-ambient critical temperature (31.1 °C) and moderate critical pressure (7.3 MPa)[10–12] and therefore, it is suitable for processing most biochemicals.[10]

Other neoteric solvents, which have received growing attention during the last few years, are ILs. Like SC CO_2, ILs has several advantages over organic solvents, since they have good chemical and thermal stability, tunable polarity, density, melting point, hydrophobicity and viscosity.[13,14] From an environmental point of view, the most important property of ILs is their negligible vapor pressure and thus they are effectively non-volatile. Additionally, ILs are able to dissolve many compounds

and can be used to form two-phase systems with many solvents in the presence of water.[15] The disadvantages of using ILs as media for enzymatic reactions are their high costs, but the good thing is that ILs are compounds which have a potential to be recycled and reused.[16] For enzyme-catalyzed reactions, ILs can be used as co-solvents in aqueous phase, as two-phase systems together with other solvents and as pure solvents.[17] In biocatalysis, ILs act as solvents, providing an adequate microenvironment for the catalytic action of the enzyme and they can be regarded as liquid immobilization supports, since multipoint enzyme-IL interactions (hydrogen, Van der Waals, ionic, etc.) may occur, resulting in a flexible supra-molecular net able to maintain active the protein conformation.[18]

Recent researches have demonstrated the possibility to carry out integral green biocatalytic processes by combining SC CO_2 and ILs with enzymes[19,20], because their different miscibilities produce the two-phase systems that show an exceptional ability to carry out both the biotransformation and the products extraction steps simultaneously.[13] Brennecke and Maginn have demonstrated that it is possible to extract a desired solute from an IL by using SC CO_2 without any contamination of the extracted solute with the IL solvent. Mainly because SC CO_2 is highly soluble in the IL phase, while the same IL are not measurably soluble in the SC CO_2 phase.[21,22] This fact not only shows the exceptional ability of SC CO_2 to extract a wide variety of hydrophophic compounds from ILs, but also decreases the viscosity of ILs, thus, improving mass-transfer phenomena.[23]

In our research transesterification of chiral substrate, (*R,S*)-1-phenylethanol, with vinyl acetate was preformed in SC CO_2 and in SC CO_2/IL biphasic media. The racemates containing (*R*)- and (*S*)-enantiomers can be differentiated by the chiral environment at the active sites of enzyme. One enantiomer fits well into the active site than its counterpart. Lipases accept a broad structural range of substrates, while retaining high enantioselectivity for each.[24,25] Although lipases are widely used as enantioselective catalyst, the structural basis for this property is unknown. The crystal structures of lipases show a large variation in the shapes of active sites.[26] This variation may explain the difference in substrate specificity. Immobilized lipase B from *Candida antarctica* (Novozym 435) has been shown to be an excellent chiral biocatalyst for the stereo-selective acylation of racemic alcohols[27] due to its very high kinetic resolution (*R*)-enantiomer yields and selectivity.[28–30]

In the researches published to date, the optimization of different reaction parameters (biocatalyst concentration, temperature, substrate concentration …) for enzyme-catalyzed transesterification of (*R,S*)-1-phenylethanol have been studied particularly in organic solvents at atmospheric pressure. Eckstein et al.[31] studied the influence of temperature on acylation of (*R,S*)-1-phenylethanol with vinyl acetate in 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl)]amide at atmospheric pressure. Some researches have been performed in SC CO_2, as well. For example, Overmeyer et al.[28] studied the influence of temperature and pressure on transesterification of 1-phenylethanol. In their case the reaction rate increased with increasing the pressure from 10 to 15 MPa at 60 °C and the optimal reaction rate was reached between 15 and 20 MPa. The Lozano's group has reported several studies based on kinetic resolution of rac-1-phenylethanol with vinyl propionate in SC CO_2/ILs biphasic systems, where they studied the influence of different ILs on CALB activity in 10 mL cartridge of an ISCO 220SX high-pressure extraction apparatus.[19,23,32–34]

This paper is focused on the preparation of chiral pharmaceutical intermediates by an enzyme-catalyzed resolution processes in SC CO_2 and in SC CO_2/IL system. A systematic investigation and optimization on the conditions for the enzyme-catalyzed transesterification of (*R,S*)-1-phenylethanol in SC CO_2 and in SC CO_2/IL biphasic system is reported. Immobilized *Candida antarctica* lipase B was used as a chiral biocatalyst and vinyl acetate as acyl donor. ILs [bmim][BF_4] was used as co-solvent when the influence of IL concentration on reaction performance was studied.

2. Materials and Methods

2. 1. Enzymes and Chemicals

Immobilized lipase B from *Candida antarctica* (Novozym 435) was kindly donated from Novozymes (Bagsvaerd, Danska). (*R*)-1-phenylethanol and (*S*)-1-phenylethanol were supplied from Sigma-Aldrich (Saint Louis, USA). 1-Butyl-3-methylimidazolium tetrafluoroborate [bmim][BF_4] (≥97%), (*R,S*)-1-phenylethanol (≥98%), vinyl acetate (≥99%), vinyl laurate (≥99%), vinyl propionate (≥98%) and vinyl butyrate (≥99%) were supplied from Fluka (Buchs, Switzerland). Decane – Reagent Plus® (≥99%) was provided from Aldrich Chemical Co. (Diesenhofen, Germany). *n*-Heptane (≥99%) was purchased from Merck (Darmstadt, Germany). Helium 6.0 was supplied from Linde plin (Celje, Slovenia) and carbon dioxide from Messer MG (Ruše, Slovenia).

2. 2. Enzyme-catalyzed Transesterification of (*R,S*)-1-phenylethanol Performed in Supercritical Carbon Dioxide

Transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2, catalyzed with chiral biocatalyst, immobilized CALB (Figure 1), was performed in a variable-volume view cell apparatus equipped with a blade-turbine stirrer to mix the phases and with two stainless steel cartridge heaters to heat the reaction mixture (Figure 2). The cell with tunable internal volume between 30 to 60

cm^3 by means of a piston operated by a hydraulic pressurization system was designed to operate up to 75 MPa and 200 °C. Initially, the bioreactor was loaded with CALB and heated to the desired operating temperature. When desired temperature was achieved, the substrates, (*R,S*)-1-phenylethanol (25 mmol) and vinyl acetate (25 mmol), were added into the bioreactor. Finally, liquid CO_2 cooled from the supply tank was pumped into the reactor up to the working pressure. The reaction was started when desired pressure was achieved and mixing of reaction mixture was turned on. During the reaction performance enantiomerically pure compound (*R*)-1-phenylethyl acetate was formed, while the (*S*)-1-phenylethanol remained unchanged. Vinyl alcohol, which tautomerizes spontaneously and quantitatively to acetaldehyde, was generated from vinyl acetate.

The enantimers concentration during the reaction time course was monitored by using a gas chromatograph. For analysis, aliquots of reaction mixture were withdrawn into a glass vial at fixed time intervals, suspended in 0.2%

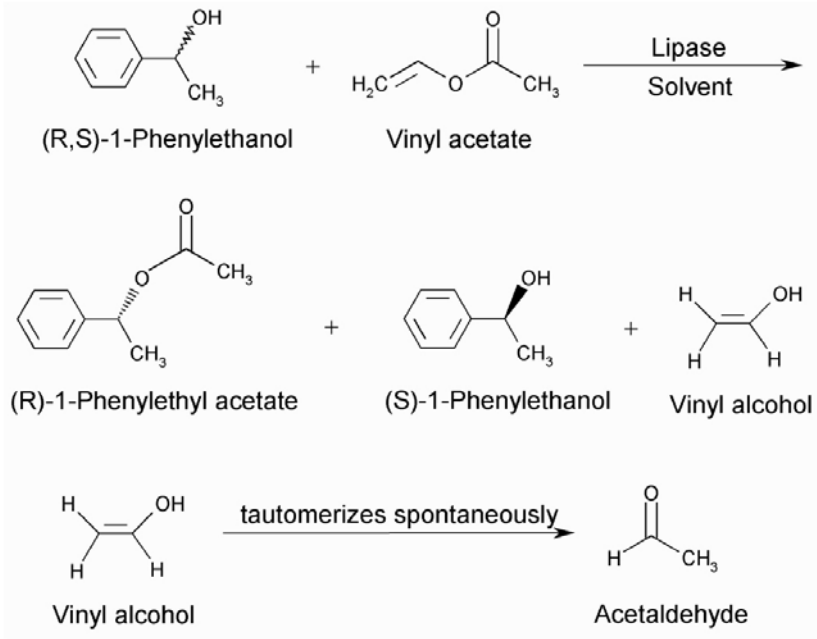

Figure 1: Schematic representation of immobilized lipase – catalyzed transesterification of (*R,S*)-1-phenylethanol with vinyl acetate as acyl donor.

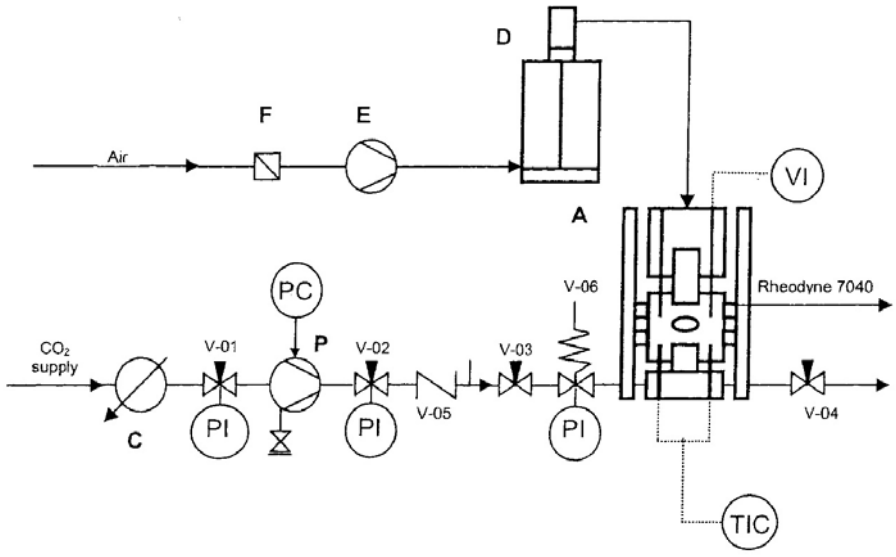

Figure 2: Sketch of the variable-volume view cell apparatus: A – variable-volume view cell; C – cooler; D – hydraulic oil system; E – air compressor; F – filter; P – high-pressure membrane pump; V01-04 – high pressure needle valves; V05 – one-way valve; V06 – safety valve; PI – pressure indicator; VI – voltage indicator; PC – pressure controller; TIC – temperature indicator controller.

solution of decane (internal standard, IS) in *n*-heptane (1 mL) and 2 μL of the resulting solution was analyzed by gas chromatograph.

2. 3. Enzyme-catalyzed Transesterification of (*R,S*)-1-phenylethanol Performed in Two-Phase System Supercritical Carbon Dioxide/Ionic Liquid

Lipase-catalyzed transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in two-phase system SC CO_2/IL, was performed in a variable-volume view cell apparatus, as well. Initially, the bioreactor was loaded with CALB (CALB/substrate ratio 11.1) and heated to the desired operating temperature. When desired temperature was achieved, the substrates, (*R,S*)-1-phenylethanol (25 mmol) and vinyl acetate (25 mmol), and certain amount of IL were added into the bioreactor. Additionally, liquid CO_2 was pumped into the reactor up to the working pressure and the reaction was started when desired pressure was achieved and mixing (300 rpm) of reaction mixture was turned on. The mechanical stirrer enabled the transfer of substrates from upper phase through [bmim][BF_4] to active sites of the CALB where the reaction took place and later the transfer of formed products from [bmim][BF_4] phase to SC CO_2 phase.

During the reaction, samples of reaction mixture were withdrawn from upper phase into a glass vial at fixed time intervals to monitor the product evolution. Figure 3 shows schematic representation of the CALB-catalyzed transesterification of (*R,S*)-1-phenylethanol in SC CO_2/[bmim][BF_4] system.

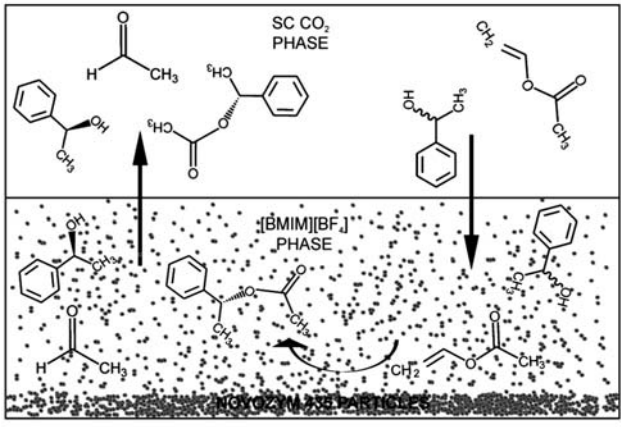

Figure 3: Schematic representation of the CALB-catalyzed transesterification of (*R,S*)-1-phenylethanol in SC CO_2/[bmim][BF_4] system.

2. 4. Gas Chromatography Analysis

Enantiomers content during the reaction time course was monitored using an HP 5890 series A gas chromatograph equipped with a flame-ionization detector (FID), using helium as carrier gas and a β-cyclodextrin capillary column (β-DEX 120) with the dimension length × I.D. 30 m × 0.25 mm with 0.25 μm film thickness (Supelco, Schnelldorf, Germany), at following temperature program: 100 °C hold for 5 min, rise up to 120 °C at rate of 5 °C/min and hold for 13 min; the temperature of injector and detector were maintained at 220 °C.

The conversion (*X*) was calculated by applying the equation, which is valid for irreversible reactions:

$$X[\%] = \frac{ee_R}{ee_R + ee_P} \times 100 .$$

At least two replicates of experiments were carried out at each operative condition. All samples, which were withdrawn from the reaction mixture, were analyzed by gas chromatograph two times. The relative deviation was evaluated to be within ± 1%.

The initial reaction rate was calculated, as well. The rate of a chemical reaction is defined as the change in the concentration of one of the reactants or products in unit time. The initial reaction rate (v_i) was determined as the slope of the tangent on the curve, which presents the concentration of produced ester [$mmol_{R\text{-}1\text{-}PEA}/g_S/g_E$, $mmol_{R\text{-}1\text{-}PEA}/g_{RM}/g_E$] in time dependence [h] in the first 10 minutes of the reaction performance, where R-1-PEA means (*R*)-1-phenylethyl acetate, S means substrates ((*R,S*)-1-phenylethanol and vinyl acetate), RM means reaction mixture and E means enzyme.

3. Results and Discussion

3. 1. Effect of Enzyme/Substrate Ratio on Transesterification of (*R,S*)-1-Phenylethanol with Vinyl Acetate in Supercritical Carbon Dioxide

Since biocatalysts are very expensive it is reasonable to optimize their concentration at first. For that purpose, influence of CALB/substrate ratio on the transesterification of (*R,S*)-1-phenylethanol was optimized. Reactions were performed to determine the minimum concentration of immobilized CALB that maximizes the concentration of formed product during the reaction performance. Experiments were conducted varying immobilized CALB/substrate ratio in the range from 4.3 to 19.9 at 40 °C and 10 MPa, with equimolar ratio of (*R,S*)-1-phenylethanol and vinyl acetate.

The influence of CALB/substrate ratio on conversion of (*R*)-1-phenylethylethanol to (*R*)-1-phenylethyl acetate is shown in Figure 4.

In order to optimize the enzyme/substrate ratio, four different CALB/substrate ratios were used for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate. As expected, the conversion of (*R*)-1-phenylethanol increa-

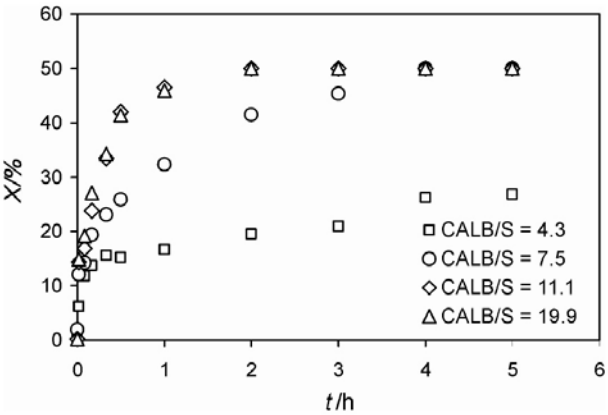

Figure 4: Influence of CALB/substrate ratio on transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, 40 °C, 10 MPa, 300 rpm, 5 h of reaction performance.

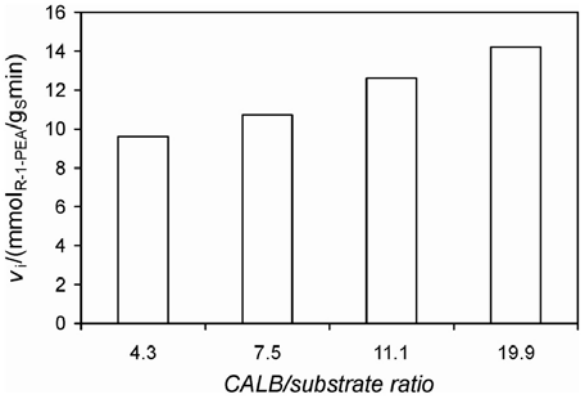

Figure 5: Influence of CALB/substrate ratio on initial reaction rate for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, 40 °C, 10 MPa, 300 rpm.

sed with increasing the CALB/substrate ratio. At lower CALB/substrate ratios the insufficient amount of the enzyme was presented and therefore the reaction proceeded slower than when the CALB/substrate ratios were higher. In the first case not enough enzymes was present for all of the reactant molecules and therefore only small part of (*R*)-1-phenylethanol could react with enzyme at the same time. When CALB/substrate ratio 4.3 was used only 19.5% conversion was attained after 2 h of reaction performance and 26.8 after 5 h of reaction performance. But, when the reaction was performed with CALB/substrate ratio 11.1 and 19.9, 50% conversion was achieved after only 2 h of reaction performance.

By increasing the amount of the immobilized CALB the initial reaction rate increased, as well (Figure 5).

The highest initial reaction rate was achieved in the case when CALB/substrate ratio 19.9 was added to the reaction mixture. At lower CALB/substrate ratios initial reaction rates decreased.

Even though higher reaction rate and 50% conversion were obtained with CALB/substrate ratio of 19.9, all further studies were done with CALB/substrate ratio of 11.1 due to high conversion attained after 2 h of reaction performance and due to problems related with sampling since the reaction mixture was saturated with enzyme.

3. 2. Effect of Temperature and Pressure on Transesterification of (*R,S*)-1-Phenylethanol with Vinyl Acetate in Supercritical Carbon Dioxide

The solubility of substrates in SC CO_2 depends on CO_2 density, which can be changed by small changes in temperature and/or pressure particularly near the critical point.[11,35] Normally, higher solubility of substances in SC

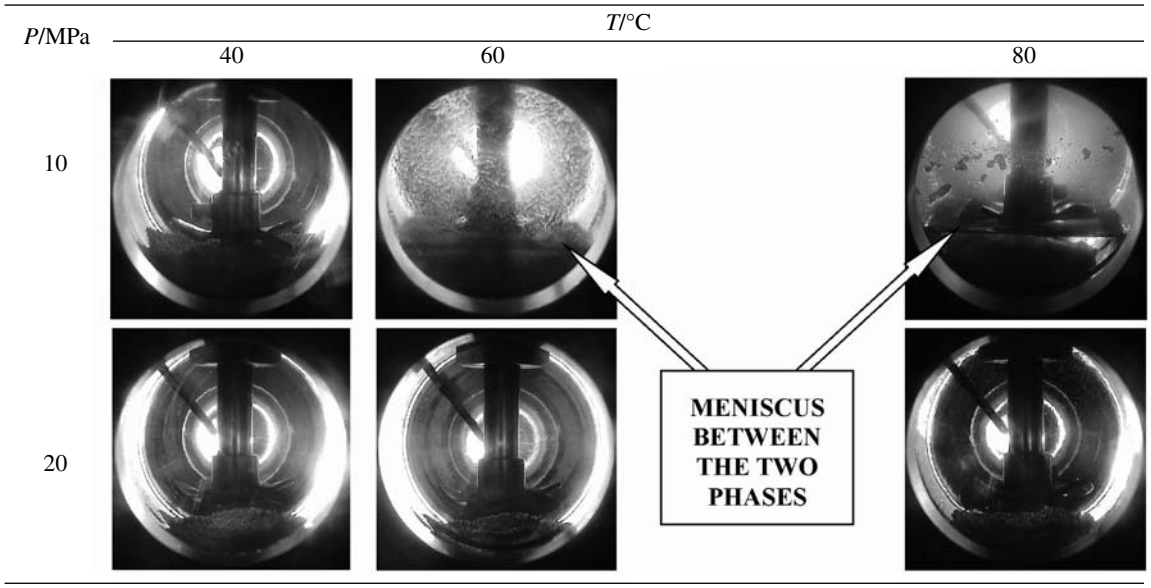

Figure 6: Phase behavior for (*R,S*)-1-phenylethanol/vinyl acetate/SC CO_2 system at different temperatures and pressures.

CO_2 is achieved with an increase in temperature. On the other hand, enzyme deactivation may occur at too high temperature. For these reasons optimal temperature for the enzyme activity and the one for the reaction performance are not necessarily the same.[36]

Before studying the temperature and pressure effect on the CALB-catalyzed transesterification of (*R,S*)-1-phenylethanol in SC CO_2, the phase behavior for (*R,S*)-1-phenylethanol/vinyl acetate/SC CO_2 system at temperatures between 40 °C and 80 °C and at 10 MPa and 20 MPa was observed visually in a view cell. The results are depicted in Figure 6.

The reaction mixture (substrates and CO_2) can be seen as one-phase system (supercritical phase) at all observed pressures at 40 °C, while at higher temperatures (60 °C and 80 °C) and 10 MPa, the reaction mixture contained both liquid and gas phase with a distinct meniscus between the two phases. By increasing the pressure from 10 MPa to 20 MPa, one-phase system was attained also for 60 °C and 80 °C. At this point, the reaction mixture has become supercritical.

On the basis of the results obtained by investigating the phase behavior of (*R,S*)-1-phenylethanol/vinyl acetate/SC CO_2 system at different temperatures and pressures, the temperature effect on CALB-catalyzed transesterification was studied in the temperature range between 40 °C and 120 °C at 20 MPa (Figure 7).

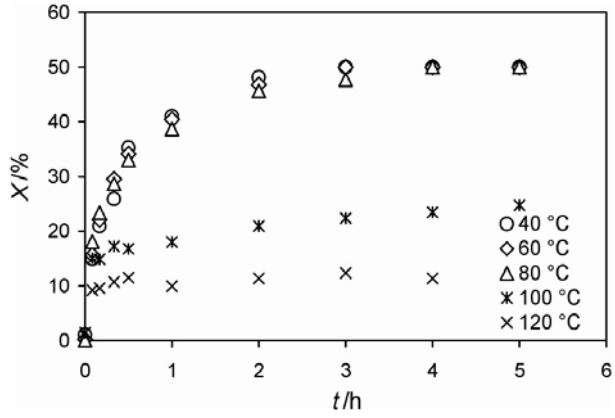

Figure 7: Influence of temperature on transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, CALB/substrate ratio 11.1, 20 MPa, 300 rpm, 5 h of reaction performance.

At temperatures between 40 °C and 80 °C there was almost no difference in reaction performance. At temperatures of 40 °C and 60 °C 50% conversion was obtained after 3 h of reaction performance and at temperature of 80 °C after 4 h of reaction. At higher temperatures (100 °C and 120 °C) the conversion drastically decreased due to thermal deactivation of CALB. As the temperature increases, molecular motion increases resulting in more molecular collisions. If, however, the temperature rises above a certain point, the heat will denature the enzyme, causing it to lose its three-dimensional functional shape by denaturing its hydrogen bonds.[37] On the other hand, the lipase preparation Novozym 435 showed some thermo resistance at temperatures up to 120 °C, since the CALB showed some activity even at 120 °C. This could be due to the immobilization on resin, which causes an increase in enzyme rigidity and therefore stability towards thermal denaturation. Moreover, lipase thermo resistance may be induced by enzyme microenvironment, which prevents unfolding of enzyme structure, since the reaction was assayed in non aqueous conditions in SC CO_2.

The temperature influenced initial reaction rates as well, which is shown in Figure 8.

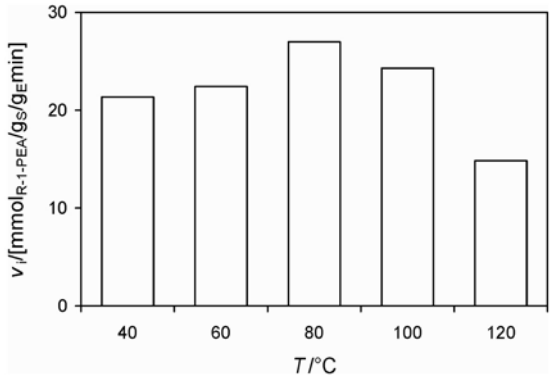

Figure 8: Influence of temperature on initial reaction rate for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, CALB/substrate ratio 11.1, 20 MPa, 300 rpm.

As expected, the initial reaction rates were found to increase with increasing temperature. The highest initial reaction rate was achieved when the reaction was performed at 80 °C. According to Overmeyer et al.[28] the physical properties of the solvent may be favorable at higher temperatures due to lower mass transfer limitations, although the enzyme activity decreases due to thermo inactivation with increasing temperatures. In our case, at temperatures above 80 °C, initial reaction rate decreased probably due to CALB thermal deactivation. Another reason for slower reaction rate at temperatures above 80 °C could be the fact that the vinyl acetate boiling point is at 73 °C and it was evaporating when it was added into the heated reactor. In the way some amount of acyl donor needed for the transesterification reaction was lost and consecutively the reaction was slower.

These results are in agreement with published data on the kinetic resolution of (*R,S*)-1-phenylethanol in SC CO_2, catalyzed with immobilized lipase B from *Candida antarctica*.[28] Authors studied the influence of temperature on synthesis of (*R*)-1-phenylethyl acetate in the tempera-

ture range from 40 °C to 160 °C and discovered that the optimal temperature for mentioned reaction was 92 °C. At lower and higher temperature they obtained lower relative initial activity.

Another parameter which can influence enzyme activity is pressure. It can modify the catalytic behavior of biocatalyst by changing for example, the rate-limiting step or modulating the selectivity of the enzyme. In most cases a pressure increase acts positive for enzymatic reactions or there are no changes in the reaction rates. Pressure-induced deactivation of enzymes takes place mostly at pressure exceeding 150 MPa. Reversible pressure denaturation mostly occurs at pressures below 300 MPa and higher pressure needed to cause irreversible denaturation.[36]

The effect of pressure on CALB-catalyzed transestserification of (*R,S*)-1-phenylethanol was studied by varying the pressure in the range from 8 MPa to 30 MPa at temperature of 40 °C, loading an (*R,S*)-1-phenylethanol/vinyl acetate in molar ratio 1:1, with CALB concentration of 10% (w/w substrate) and stirrer rate of 300 rpm. The visual observations of the phenomena that occurred during the experiments showed that the supercritical reaction mixture was attained at all tested pressures. The obtained results are illustrated in Figure 9.

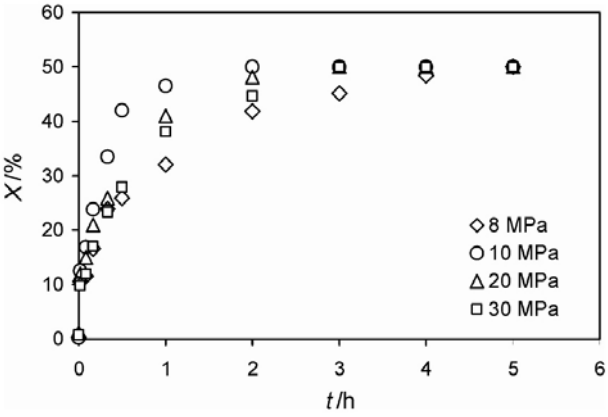

Figure 9: Influence of pressure on transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, CALB/substrate ratio 11.1, 40 °C, 300 rpm, 5 h of reaction performance.

As can be seen from Figure 9, the reaction performance depends on pressure. Even though the obtained conversion was 50% after 5 h of reaction performance for all tested pressures, biggest differences can be observed in the first 2 h of reaction. Apparently, the conversion increased by increasing the pressure from 8 MPa to 10 MPa, which could be due to the fact that an increase in pressure leads to enhanced solvent density, improving its solvating power in the reaction bulk.[35] Further increase in pressure to 20 MPa or 30 MPa resulted in decrease in conversion. This could be connected with the dilution effect of the substrates caused by larger amount of CO_2 at higher pressures, which consecutively increased the reaction volume.[38]

The influence of pressure on initial reaction rate is shown in Figure 10.

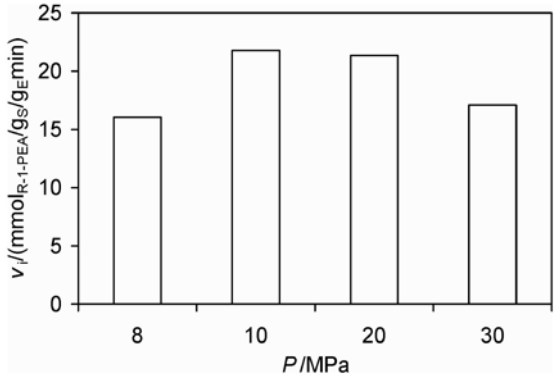

Figure 10: Influence of pressure on initial reaction rate for transesterification of (*R,S*)-1-phenylethanol with vinyl acetate in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol vinyl acetate, CALB/substrate ratio 11.1, 40 °C, 300 rpm.

The highest initial reaction rate was obtained when the reaction was performed at 10 MPa. At lower and higher pressure the reaction rate decreased. By increasing the pressure the solubility of substrates in SC CO_2 increases and consecutively the initial reaction rate is higher. On the other hand, high pressure may cause changes in enzyme conformation which leads to lower enzyme activity.[39] On the contrary, authors Overmeyer at al.[28], who studied the influence of pressure on lipase-catalyzed kinetic resolution of (*R,S*)-1-phenylethanol at 60 °C, obtained the highest lipase activity at pressures between 15 MPa and 20 MPa.

3. 3. Effect of Acyl Donor on Transesterification of (*R,S*)-1-Phenylethanol in Supercritical Carbon Dioxide

The common acyl donors for the enzymatic resolution of racemic alcohols are carboxylic acids, anhydrides, vinyl ester, diketene and ester. Each class of acyl donors produces different types of by-products. The effects of the by-products on the reactions are the main consideration in the acyl donor selection process.[40]

Enol esters are the most used as regards kinetic resolutions of racemic alcohols.[19,28,41–45] They liberate unstable enols that tautomerizes to ketones or aldehydes, thus making the reaction irreversible. Aldehydes have been shown to deactivate some lipases by forming Schiff's bases with exposed lysine residues, but CALB has proven to be little affected.[46,47] A large variety of vinyl esters are commercially available which makes it easy to optimize the acyl-chain length of the acyl-donor to be used with the alcohol of interest.[40,42]

Three vinyl esters with different chain length (vinyl acetate, vinyl butyrate and vinyl laurate) were used at molar ratio of acyl donor/(*R,S*)-1-phenylethanol of 1 : 1 in SC CO_2 at 40 °C and 10 MPa.

Influence of vinyl esters with different chain length as acyl donors on the conversion is shown in Figure 11.

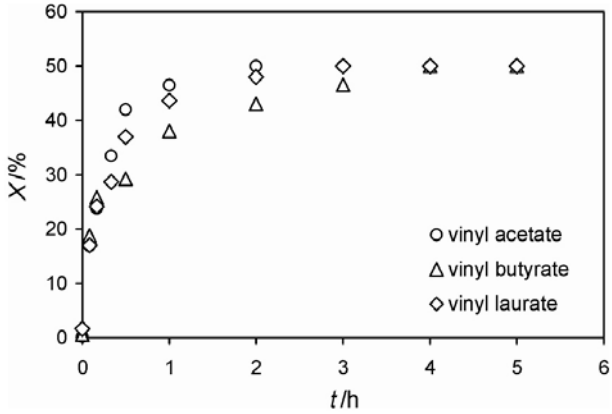

Figure 11: Influence of acyl donor on transesterification of (*R,S*)-1-phenylethanol in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol acyl donor, CALB/substrate ratio 11.1, 40 °C, 10 MPa, 300 rpm, 5 h of reaction performance.

Using vinyl esters, such as vinyl acetate, vinyl butyrate and vinyl laurate as acyl donors for transesterification of (*R,S*)-1-phenylethanol, 50% conversion was obtained after 5 h of reaction performance. Biggest differences in reaction performance were observed in the first part of the reaction (in the first 3 h of reaction performance). Although, 50% conversion was obtained after 4 h of reaction performance when vinyl butyrate was used as acyl donor, while with vinyl acetate 50% conversion was achieved after just 2 h, the highest initial reaction rate was achieved with vinyl butyrate (Figure 12). That could be related to specificity of lipases. Regiospecificity of lipases may be responsible for enhanced reactivity towards long carbon chain fatty acids.[48,49] On the other hand, with higher vinyl ester, vinyl laurate, the lowest initial reaction rate was attained. Analogously to the lipase-catalyzed resolution of 5-phenyl-1-pentan-3-ol, vinyl acrylate and vinyl crotonate reacted slower than vinyl acetate. This is described to the steric hindrance owing to the comparative inflexibility of the α,β-alkenic ester.[42]

The effect of different acyl donors on acylation of (*R,S*)-1-phenylethanol was studied by Bakker et al.,[42] as well. They tested 12 different acyl donors, including vinyl acetate, vinyl propionate and vinyl laurate, for the acylation of 1-phenylethanol in *n*-hexane at atmospheric pressure. In their case the highest initial reaction rate was obtained with vinyl butyrate and the lowest with vinyl laurate. In researches published to date, different vinyl esters have not been screend for transesterification of (*R,S*)-1-phenylethanol in SC CO_2.

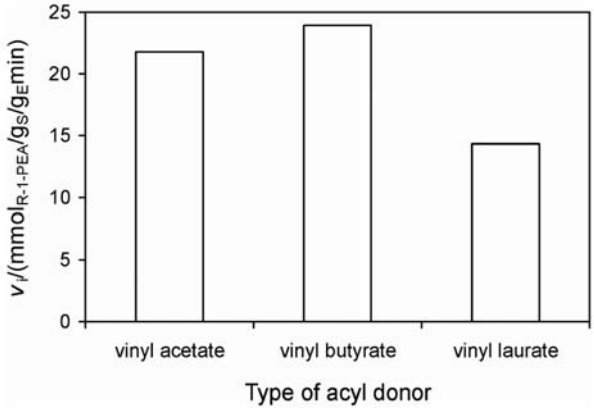

Figure 12: Influence of acyl donor on initial reaction rate for transesterification of (*R,S*)-1-phenylethanol in SC CO_2. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol acyl donor, CALB/substrate ratio 11.1, 40 °C, 10 MPa, 300 rpm.

3. 4. CALB-Catalyzed Transesterification of (*R,S*)-1-Phenylethanol in Supercritical Carbon Dioxide/Ionic Liquid System

CALB-catalyzed transesterification of (*R,S*)-1-phenylethanol was performed in SC CO_2/[bmim][BF_4] system, where the effect of [bmim][BF_4] concentration on lipase activity was studied. Reactions were performed with IL concentrations between 0 mmol to 50 mmol, with molar ratio of substrates, (*R,S*)-1-phenylethanol and vinyl acetate, of 1:1 at 40 °C and 10 MPa.

Since SC CO_2 dissolves quite well in ILs, but ILs do

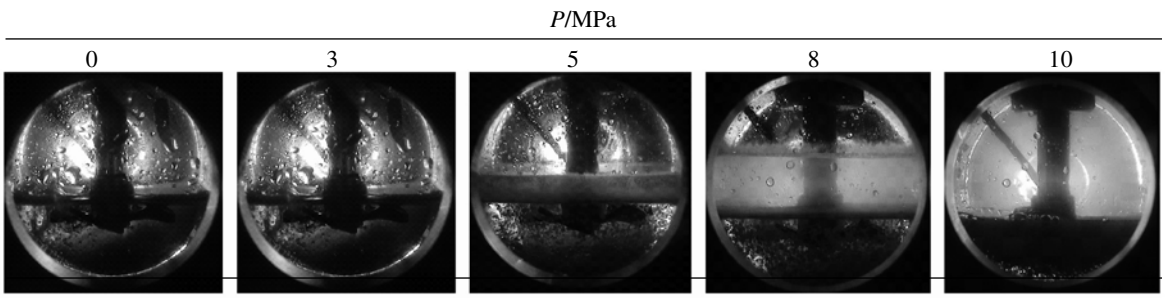

Figure 13: Visual vapor-liquid phase equilibrium observations of the reaction bulk at 40 °C and different pressures.

not dissolve in carbon dioxide,[50] two-phase system was obtained when the reaction was carried out in $SCCO_2$/[bmim][BF_4] at 40 °C and 10 MPa (Figure 13).

In Figure 14 is shown how the phase behavour for (*R,S*)-1-phenylethanol/vinyl acetate/SC CO_2/[bmim][BF_4] system was changed as the pressure was increased from 0 MPa to 10 MPa at 40 °C. At 0 MPa only liquid reaction bulk (solution of (*R,S*)-1-phenylethanol and vinyl acetate) with CALB particles was attained. Addition of CO_2 in the system leaded to form a two-phase system. CALB particles were suspended in lower phase (catalytic phase), which was rich with [bmim][BF_4], while the upper phase was rich with CO_2 and substrates.

The effect of [bmim][BF_4] on CALB activity at 40 °C and 10 MPa is shown in Figure 14.

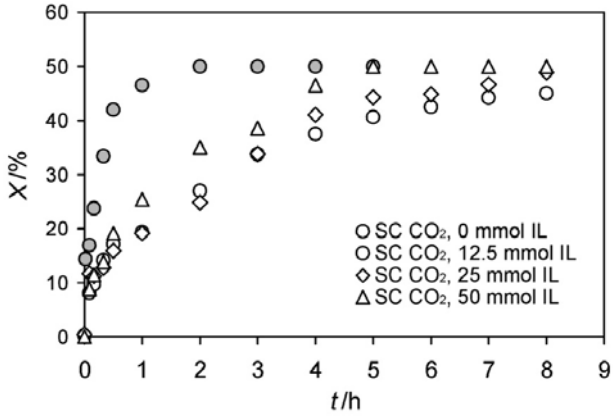

Figure 14: Influence of [bmim][BF_4] concentration on transesterification of (*R,S*)-1-phenylethanol in SC CO_2/IL system. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol acyl donor, CALB/substrate ratio 11.1, 40 °C, 10 MPa, 300 rpm, 8 h of reaction performance.

CALB activity was tested with [bmim][BF_4] concentrations between 0 mmol and 50 mmol. The highest CALB activity was observed when the reaction was performed in system without [bmim][BF_4], where 50% conversion was obtained after 2 h of reaction performance. The results showed that the introduction of [bmim][BF_4] into the reaction mixture influenced the reaction performance, which could be related to the presence of external mass transfer limitations since the reaction was performed in two-phase system instead of in one-phase system like when the reaction was performed without [bmim][BF_4]. Namely, CALB particles were suspended in [bmim][BF_4] phase, while the substrates were in the supercritical phase. Firstly, the molecules of substrates had to transfer from SC CO_2 phase to [bmim][BF_4] phase towards CALB particles and secondly, products had to be transfered back to SC CO_2 phase. Nevertheless, the best results were obtained when the highest amount of [bmim][BF_4] (50 mmol) was used for the transesterification.

The initial reaction rates were affected by [bmim][BF_4] concentration, as well (Figure 15). They were increasing with increased IL concentration. Since ILs are good solvents for many organic compounds,[14] higher IL concentration in the reaction mixture could cause higher solubility of substrates[51] and consecutively higher conversion and initial reaction rate were obtained.

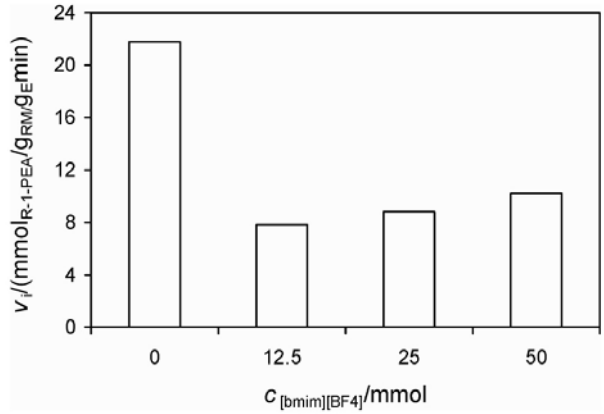

Figure 15: Influence of [bmim][BF_4] concentration on initial reaction rate for transesterification of (*R,S*)-1-phenylethanol in SC CO_2/IL system. Reaction conditions: 25 mmol (*R,S*)-1-phenylethanol, 25 mmol acyl donor, CALB/substrate ratio 11.1, 40 °C, 10 MPa, 300 rpm.

Authors Nara et al.[52] studied the influence of 1-butyl-3-methylimidazolium hexafluorophosphate [bmim][PF_6] concentration on lipase-catalyzed transesterification of 2-hydroxymethyl-1,4-benzodioxane with vinyl acetate. Experiments, wherein [bmim][PF_6] was added as an additive to the solvent dichloromethane, were performed with 0 to 90% v/v of [bmim][PF_6] in the reaction mixture. Their study revealed that as the proportion of [bmim][PF_6] was increased, a slight increase in initial rates was observed. Furthermore, authors Miyawaki and Tatsuno[53] studied the influence of methyltrioctylammonium trifluoroacetate [mtoa][tfa] concentration on lipase-catalyzed butanolysis of triolein. Once again, the best results were obtained when the highest amount of IL was added to the reaction mixture (80% of [mtoa][tfa]). In their case, a small amount of IL added seemed inhibitory, while the large amount of IL added accelerated the reaction in spite of the reduction in substrate concentration.

4. Conclusion

Chiral phenylethanol derivatives are important chiral building blocks and synthetic intermediates for pharmaceuticals, agrochemicals and natural products.[51] One approach to satisfy the increased demand for production of single enantiomers of chiral intermediates is enzymatic synthesis in non-aqueous media. Therefore, an appropriate reaction medium for the biosynthesis has to be selected. SC CO_2 and ILs are promising media for enzymatic reac-

tions. In the present study, the potential utilization of SC CO_2 and SC CO_2/IL system was investigated for the transesterification of chiral alcohol, (*R,S*)-1-phenylethanol, where *Candida antarctica* lipase B was used as chiral biocatalyst and vinyl acetate as the acyl donor. The effect of CALB/substrate ratio, temperature, pressure, the type of vinyl ester and [bmim][BF_4] concentration on the catalytic efficiency of CALB was evaluated.

At CALB/substrate ratio 19.9 at 40 °C and 10 MPa, using an equimolar ratio of (*R,S*)-1-phenylethanol/vinyl acetate after 2 hours of bioconversion, the highest possible conversion (50%) was reached. The same conversion yield was attained when temperature and pressure effect was studied. Although, the optimal CALB/substrate ratio, temperature and pressure, were determined to be 19.9, 80 °C and 10 MPa, respectively, all further experiments were performed with CALB/substrate ratio 11.1 at 40 °C and 10 MPa due to easier sampling of reaction mixture during the reaction performance and easier reaction mixture preparation, since at higher temperatures the vinyl acetate was evaporating. When different vinyl esters were used as acyl donor for abovementioned reaction, the highest initial reaction rate was obtained with vinyl butyrate.

Enzymatic synthesis in SC CO_2 and SC CO_2/IL media proved to be an affective preparative method for the synthesis of single enantiomer of chiral intermediates applicable in pharmaceutical industry.

5. Acknowledgements

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Povzetek

Komercialno imobilizirano lipazo B iz *Candida antarctica* (CALB) smo uspešno uporabili kot biokatalizator za encimsko katalizirano transesterifikacijo (*R,S*)-1-feniletanola v SC CO_2 in v dvo-faznem sistemu SC CO_2/IL. Najprej smo proučili vpliv različnih reakcijskih parametrov, kot so razmerje CALB/substrati, temperatura in tlak, na aktivnost CALB v SC CO_2. Z naraščanjem razmerja CALB/substrati od 4.3 do 19.9, temperature od 40 °C do 80 °C in tlaka od 10 MPa do 30 MPa so naraščale presnove in začetne reakcijske hitrosti. Nadaljnje naraščanje temperature od 80 °C do 120 °C in tlaka od 20 MPa do 30 MPa je povzročilo znižanje tako presnove kot začetne reakcijske hitrosti. Nadalje smo za transesterifikacijo (*R,S*)-1-feniletanola v SC CO_2, katalizirano z imobilizirano CALB, uporabili kot donorje acilne skupine vinil estre z verigami različnih dolžin. Najvišjo začetno reakcijsko hitrost smo dosegli z vinil butiratom, čeprav smo 50% presnovo hitreje dosegli z vinil acetatom.
V dvo-faznem sistemu SC CO_2/IL smo proučili vpliv koncentracije ionske tekočine 1-butil-3-metilimidazolijevega tetrafluoroborata [bmim][BF_4] na potek reakcije. Najvišjo začetno hitrost reakcije smo dosegli, ko smo reakcijo izvedli s 50 mmol (70% w/w reakcijske zmesi) [bmim][BF_4].